

THE DEVELOPMENT ON, AND PENETRATION INTO PINUS SYLVESTRIS FOLIAGE, OF A

PATHOGENIC LOPHODERMUM

J. M. STALEY

Rocky Mountain Forest and Range Experiment Station, USDA, 240 West Prospect Street, Fort Collins, Colorado 80521, U.S.A.

A Lophodermum distinct from Pinastri attacks Pinus sylvestris in nurseries and Christmas tree plantings in the Pacific Northwestern USA. It appears to be the same fungus reported as Lophodermum pinastri from Wisconsin, Michigan, Pennsylvania, and New York. It is unclear whether it is the same fungus reported from North Carolina and Georgia as L. pinastri.

The species is distinguished by: subepidermal development, conidial size, cultural characters, and gross appearance, from L. pinastri. In some collections a faint greenish tinge can be seen in the Tabia. Such tabia become yellow if preserved by drying for an extended period. The fungus is also distinguished from L. pinastri by its association with prominent late winter and early spring reddening of current internode and older needles.

In addition to the histological evidence of direct penetration, there is abundant and impressive evidence that the fungus is an aggressive pathogen. The fungus develops on nursery seedlings. It is carried into plantings where it intensifies over a several-year period, and after 5-7 years blossoms as the trees close up into a dramatic symptomatic display. Control is readily obtained by chemicals in the nursery, and almost surprisingly a planting in a severely infected condition can be sprayed for three years with almost complete remedial effect at a cost of 2 cents/tree/year, which cost includes machinery, gasoline, oil, operator, and chemicals.

Spore deposition, germination, development on, and penetration of the leaf surface were studied in an infected stand near Rochester, Washington. Infection here was severe. The pathogen left few older needles on which other Lophodermia might fruit. Spore deposits onto ungreased glass slides exposed for two weeks were as high as 2200 spores per cm<sup>2</sup>. This was a more dense deposition than had been recorded at another severely infected site as shown here in green for the year 1972. In 1973 ascospore dispersal began earlier, about September 6, and continued intermittently until December. Ascospores could not definitely be identified to species so my identification of the germinating spores depends on the circumstances of the study, that is: the apparent absence of other Lophodermia, the absence of older foliage that would support other Lophodermia, the timing of observations to avoid sporulation periods of other Lophodermia, and the abundance of spore dispersal of my target fungus.

Spores suspended in water droplets on a glass slide were observed to sink, but they did not adhere to the glass surface as long as the water remained. Within one second after evaporation of the water the spore was fastened so securely to the glass that it could not be moved without rupturing. It seems likely, from this, that spores are free to move about on wet pine needles.

In fact they do lodge in indentations between epidermal cells more frequently than would otherwise be expected. Deposition is most frequent on that distal portion of the needle not protected by needles attached below, however deposition and adhesion were frequently seen at any random location on the needle. Needles to be studied were coated with a 25 percent dilution of Duco plastic cement in benzene. Observations were occasionally compared using other cements and solvents. The resulting impressions revealed outlines of epidermal cells, stomatal openings, and leaf flora in various stages of development. Other mounting media were used at times, but the mounts used routinely were Orsiellin BB - Lactophenol (or Polyvinyl Lactophenol).

Following deposition first the germ tube is formed. It enlarges into a hyaline appressorium with relatively thin walls. Germination and hyaline appressorial formation commonly occurred in over 50 percent of spores trapped on clean glass slides exposed one week. On the leaf surface shown here, however, melanization of appressoria was noted 3-4 weeks after spore dispersal began.

Melanization of appressoria was studied on untreated leaf surfaces and compared with appressorial melanization on leaf surfaces sprayed with fungicide. Over 80 percent of the appressoria melanized in the absence of fungicide. Less than 5 percent melanization was found in the presence of the most effective fungicides. Symptom development was prevented by the fungicides. This suggests that melanization is a necessary step in the penetration of the needle.

Examination of sections cut from the distal 1/3 of the needle revealed frequent penetration from melanized appressoria. Penetration hyphae grew directly through the cuticle and epidermis. In no case was penetration through the stomatal opening, even when appressoria formed nearby. The average distance from appressoria to stomatal openings was over  $20\mu$ , further than the distance grown in development of appressoria. Germination hyphae growing freely over the leaf surface or on exposed glass slides were not observed. Penetration of the leaf was observed away from stomatal openings, however direct penetration near a stoma did lead in at least one instance to hyphal growth in the substomatal chamber.

Spore dispersal was initiated in early September. Penetration was not observed until December. However symptom development was evident by late October and prominent in November. It appears that appressorial melanization begins 2-4 weeks after spore deposition, and that penetration proceeds shortly thereafter. It is hoped that a knowledge of the process will allow further study to measure the frequency of these sequential events with time.

Following melanization of the appressoria the ascocarp itself may weather away, leaving only a structure heretofore not recognized as part of the infection process. They are often characteristically lobed, resembling somewhat the melanized appressoria reported for Colletotrichum by SUTTON. They possess an internal structure that is distinctive and may indicate the point of penetration. They can, with experience, be distinguished from similar objects such as these unidentified ascospores which were commonly deposited on the pine needles studied.

### Summary

A Lophodermium species distinct from L. pinastri is capable of attacking current year (and older) foliage of Pinus sylvestris. In western Oregon and Washington it casts its spores largely in the autumn. Ascospores adhere to dry (but not to wet) surfaces of microscope slides (and presumably to leaf surfaces). They appear to lodge at irregularities on wet needle surfaces, a favoured location being at the edge of subsidiary cells near stomata. After germination and appressorial formation, melanization of the appressoria occurs. Since appressorial melanization and symptom development are largely prevented by fungicides, melanization is believed requisite to penetration. Penetration is directly through the cuticle and epidermis by means of penetration hyphae, even when spores lodge in the vicinity of stomata. The pathogen is readily controlled by maneb, benlate, or fundalin. Since it appears to develop under nursery culture and is carried on nursery seedlings to the field plantings where it intensifies over a 4-8 year period, there is hope that the disease can be controlled by nursery sanitation.

### Zusammenfassung

#### Die Entwicklung einer pathogenen Lophodermium-Art auf und das Eindringen in Nadeln von Pinus sylvestris

Eine von Lophodermium pinastri deutlich zu trennende Lophodermium-Art vermag einjährige (und ältere) Nadeln von Pinus sylvestris zu befallen. Im westlichen Oregon und Washington findet der Sporenflug größtenteils im Herbst statt. Die Ascosporen heften sich an trockene, jedoch nicht an nasse Oberflächen von Objekträgern und vermutlich auch an Nadeloberflächen an. Sie scheinen auf nassen Nadeln an Unregelmäßigkeiten der Oberfläche hängen zu bleiben, bevorzugt an den subsidiären Zellen in Nähe der Stomata. Nach Keimung und Appressorienbildung erfolgt eine Melanisierung der Appressorien. Da die Appressorienmelanisierung und die Symptomentwicklung durch Fungicide weitgehend verhindert werden, kann man die Melanisierung als eine Voraussetzung für das Eindringen betrachten. Das Eindringen erfolgt mit Hilfe von Penetrationshyphen direkt durch Cuticula und Epidermis, selbst dort wo die Sporen neben Stomata liegen. Der Erreger ist durch Fungicide wie Maneb, Benlate oder Fundalin einfach zu bekämpfen. Da er sich offenbar während der Baumschulkultur entwickelt und mit den Sämlingen in Feldpflanzungen verschleppt wird, wo sich der Befall im Verlauf von 4-8 Jahren intensiviert, besteht die Hoffnung, die Krankheit durch sanitäre Maßnahmen in der Baumschule bekämpfen zu können.